

INDUCTION OF PROGRESSIVE NEPHROPATHY IN RATS BY ELUTED γ -GLOBULIN AND FREUND'S ADJUVANT

By **A. H. NAGI, M.B., B.S., Ph.D.**

Department of Pathology, Royal Victoria Hospital, Belfast

INTRODUCTION

IT has been suggested that anti-basement membrane (BM) antibody is responsible for the development of the pulmonary and the renal lesions in Goodpasture's syndrome (Sturgill and Westervelt, 1965; Beirne, et al, 1968; Willoughby and Dixon, 1970). It is a controversial issue whether this anti-BM antibody is formed by the release of glomerular or alveolar BM antigen.

Experimental models resembling Goodpasture's syndrome have been produced in animals by the injections of anti-lung antiserum (Hogadorn et al, 1969; Willoughby and Dixon, 1970). It has also been reported that heterologous non-organ specific antigen (lung homogenate) can initiate an auto-immune type of glomerulonephritis (Stebly and Rudofsky, 1968).

The pathogenic role of the anti-glomerular basement membrane (GBM) antibodies in the production of glomerular lesion in Goodpasture's has also been demonstrated by a successful transfer of acute glomerulonephritis to monkeys by the intravenous injection of eluted γ -globulin from patients with Goodpasture's syndrome (Lerner et al, 1967; Lerner and Dixon, 1968b). Poskitt (1970) demonstrated the presence of γ -globulin in the GBM of mice 24 hours after the injection of a similarly eluted γ -globulin but he failed to produce a progressive glomerular lesion. Nagi et al (1971) induced progressive glomerular and pulmonary lesions in rats which were made proteinuric prior to the intravenous administration of eluted γ -globulin from a case of Goodpasture's syndrome. They concluded that serum sickness plays an important part by allowing the injected γ -globulin to come in contact with some component of the GBM.

The present experiment describes an attempt to induce progressive glomerulonephritis in Wistar rats by intraperitoneal injections of Freund's complete adjuvant and eluted γ -globulin from the kidney of a case of Goodpasture's syndrome.

MATERIALS AND METHODS

The γ -globulin was eluted from the kidney of a case of Goodpasture's syndrome with a method used by Nagi et al (1971).

Thirty-two Wistar rats were divided into the following three groups. Group A – In this group 20 rats were injected by intraperitoneal route with a homogenate of 0.2 ml of the eluted γ -globulin and 0.4 ml of Freund's complete adjuvant per rat for 6 weeks. Group B – In this group 6 rats were injected with 0.2 ml of the eluted γ -globulin in normal saline. Group C – In this group 6 rats were injected with Freund's adjuvant alone. The protein estimations were carried out on the 24 hour specimens of urine at weekly intervals till the termination of the experiment at 18 weeks. Kidney and lung tissues obtained from each rat were examined by histological, immunofluorescent and electronmicroscopical techniques.

RESULTS

During the course of the experiments the proteinuria in the control groups (B and C) of animals was within normal limits, whereas in the test group it showed continuously rising levels (Figure 1).

The results of immunofluorescent studies are summarised in Table I. It is evident that no control animals had the glomerular localisation of human or rat globulins.

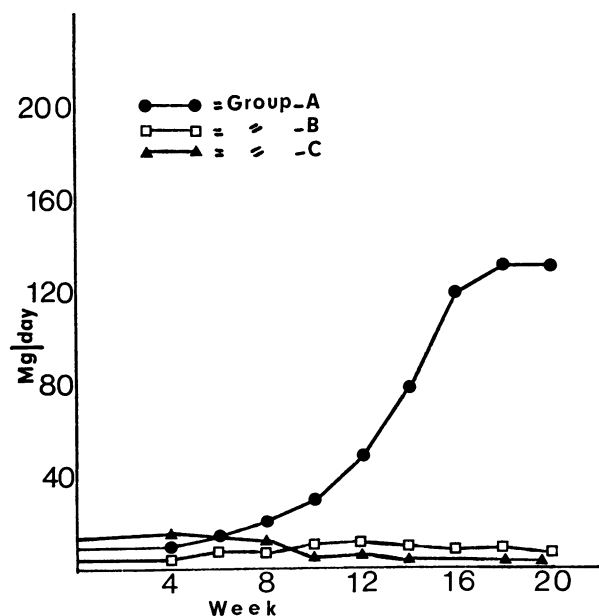


FIG. 1. Proteinuria levels in three groups of animals.

On the other hand, 16 of the 20 rats in Group A showed linear localisation of auto-logous γ -globulin and complement along the GBM and alveolar capillary BM.

In the kidneys of Group A animals moderate to severe histological abnormalities were observed by the various staining techniques. From 50 to 75 per cent. of the glomeruli in this group showed proliferation of endothelial and mesangial cell. Most of the glomeruli showed this change in 2 to 3 lobules whereas the remainder of the lobules appeared normal. The capillary lumens in many glomeruli are filled with PAS

TABLE
Immunofluorescent Studies

Group	Rat IgG	Rat BiC	Human IgG	Human BiC	No. of Animals with lesions
A	+	4/20	None	None	16/20
	++	12/20			
	+++	—			
	++++				
B	+	None	None	None	0/6
	++				
	+++				
	++++				
C	+	None	None	None	0/6
	++				
	+++				
	++++				

positive material which also stained for fibrin. The proximal convoluted tubules around the affected glomeruli were dilated and contained proteinaceous casts in their lumens (Figure 2). By electron microscopy the glomerular lesions were recognised by irregularly thickened BM and proliferation of the BM-like material towards the

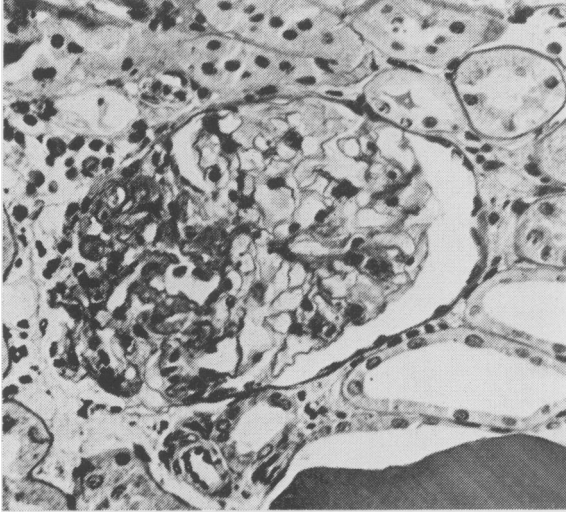


FIG. 2.

Cellular proliferation and other changes described in the text can be seen in part of the glomerulus.

the endothelial aspect. The lumens in some of the capillary loops were obliterated with this BM-like material. Most of the affected loops showed subendothelial electron dense deposits (Figures 3 and 4). The epithelial cell foot processes were fused over the affected loops. The tubules contained numerous protein droplets within their epithelial cells.

Lungs: All rats in Group-A showed moderate degree of lymphocytic infiltration of the inter-alveolar speta with some thickening of the alveolar walls. A few deposits of haemosiderin were also seen in most of the specimens.

The control groups B and C showed none of the histological, electron or immunofluorescent microscopical abnormalities.

DISCUSSION

The results of the present experiment show that eluted γ -globulin from the kidney of a patient with Goodpasture's syndrome can initiate a disease recognised by progressive lesions of lungs and kidneys. It is also evident that these lesions were consistently produced in Group-A rats which were injected with a homogenate of the eluted γ -globulin and Freund's adjuvant. Fluorescent antibody test revealed the localisation of autologous γ -globulin and complement in a focal linear distribution along the glomerular and the alveolar capillary BM. On histological and electron microscopical examination these lesions were similar to those described by Nagi et al (1971).

The pathogenesis of Goodpasture's syndrome is still a controversial issue. Various experimental models suggest that the lesions might be initiated by antibodies directed against both lungs and kidneys or anti-GBM antibodies which cross-react with alveolar capillary BM. (Stebly and Rudofsky, 1968, Hagadorn et al, 1969; Willoughby and Dixon, 1970). Lerner and Dixon (1968a) put forward the possibility that certain "undefined associated factors must be present before the GBM-antigen can induce glomerulonephritis". Nagi et al (1971) suggest that these undefined factors could be serum sickness induced changes in the GBM permeability and the presence of an excessive amount of anti-GBM antibody.

The actual mechanism by which the present experimental model is produced is not known. We put forward a hypothesis which may elucidate the events leading to the production of this disease. The vulnerability of the GBM is increased by the administration of Freund's adjuvant (Heymann et al, 1962 and 1963; Watson,

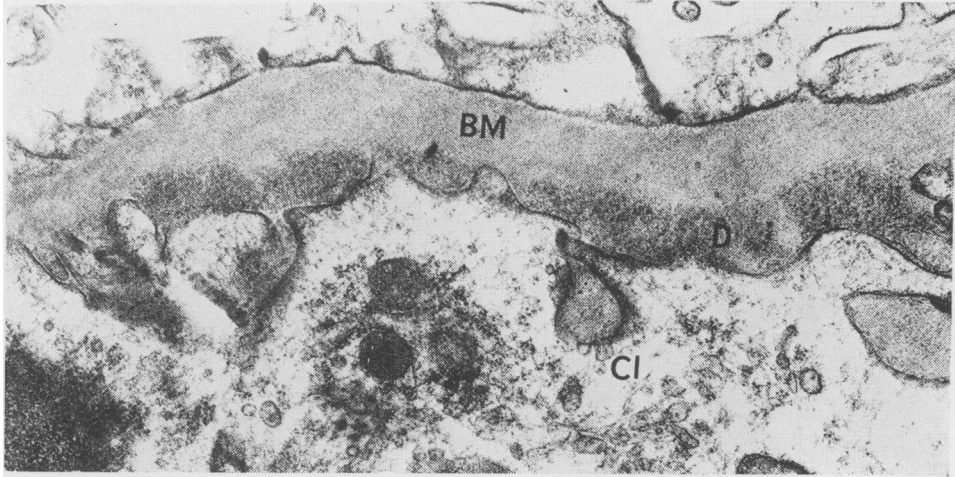


FIG. 3 (above)
Osmiophilic deposit along the sub-endothelial aspect of the thickened glomerular basement membrane in a group-A rat. Electron micrograph.



FIG. 4. (left).
Another glomerular loop showing marked proliferation of the GBM, resulting in the obliteration of its lumen.

BM = Basement mebrane
En = Endothelial cells
D = deposit
Fp = Foot processes
Cl = Capillary lumen

Dixon and Feldman, 1965; Cuppage, 1965) and by serum sickness (Barabas et al, 1970; Nagi et al, 1971). Freund's adjuvant containing eluted γ -globulin might have come into contact with the nephritogenic material in the GBM leading to its intravascular release and the production of autoantibodies. These autoantibodies might have started a cycle of events which maintained the release of the GBM antigens and the formation of autoantibodies which might result in the type of tissue damage observed in the lungs and kidneys of these rats. Autoantibodies formed against the GBM might have cross-reacted with the alveolar capillary BM resulting in the dual lesions in this disease. In view of this observation it is suggested that in addition to serum sickness certain factors (Freund's adjuvant in this case) which increase the vulnerability of the BM may also help in initiating an event responsible for the production of a progressive disease of this nature.

SUMMARY

A progressive glomerulonephritis and pulmonary lesion have been produced in Wistar rats using a homogenate of eluted γ -globulin from a case of Goodpasture's syndrome, and Freund's complete adjuvant. It is suggested that the Freund's adjuvant—induced changes in the vulnerability of the GBM may contribute to the initiation of an autoimmune cycle which may lead to the production of a progressive disease of the BM.

My thanks are due to Professor Sir J. H. Biggart, C.B.E., for his advice, Messrs. W. Kirkwood, P. Westwood and S. Cameron for their technical help, and Mr. D. Mahaffy for the photography.

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